

BCMB 8190

PROBLEM SET 9

1) In an SAR by NMR experiment two ligands, A and B, are found to perturb resonances in an HSQC experiment that belong to sites that are within 6 Å of one another. Ligand A has a dissociation constant of 2×10^{-4} and ligand B has a dissociation constant of 5×10^{-6} . If we successfully link these in such a way that their individual binding geometries are not perturbed, and entropy effects are minimal, what would you expect to find for the dissociation constant of the linked ligand?

2) We have a ligand of molecular weight 2000 that binds tightly, but with fast exchange, to a protein of molecular weight 300,000. At 600 MHz the ligand is giving negative NOEs by itself in solution. We would like to see transferred NOEs that are contaminated by no more than 20 % by NOEs from the unbound state. What is the highest ligand to protein ratio that we could safely use?

3) In an MRI experiment contrast agents are used to help resolve spatially distinct elements by selectively shortening T1 or T2 spin relaxation times for those elements. Images are usually displayed with regions giving the most signal as light areas and those giving the least signal as dark areas. Suppose we use an iron oxide nanoparticle as a contrast agent. Would you use a T2 enhanced or T1 enhanced sequence to acquire data? Would the areas affected by the nanoparticle appear as dark or light areas?

4) Suppose we observe a reduction in spin lattice relaxation times (sec) for H8 protons on G7, A8, and A9 of a nucleic acid double helix on substituting Mn(2+) for Mg(2+) as follows: G7 from 2.0 to 1.5, A8 from 2.0 to 0.5, and A9 from 2.0 to 1.5. Assuming the protons are 5 Å apart and on a straight line, how far is the binding site from each proton?

5) Estimate the rates of chemical exchange for the following examples:

a) The NMR spectrum of N,N-dimethylnitrosamine shows distinct cis and trans methyl lines with width at half height = 1 Hz and a separation of 100 Hz. The inherent resolution due to homogeneity is 0.5 Hz.

b) The same molecule in the same spectrometer shows a single line of width 5 Hz.